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EFFECT OF HEMATIN ON THE RECOVERY OF BACILLUS ANTHRACIS AND RELATED ORGANISMS

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ABSTRACT

Freshly prepared alkaline hematin inhibits the growth of many Gram-positive organisms, including *Bacillus anthracis*; Gram-negative organisms, however, are generally not inhibited. Work on a selective medium for isolating *B. anthracis* that does not contain hematin as a selective ingredient is in progress.

EFFECT OF HEMATIN ON THE RECOVERY OF *BACILLUS ANTHRACIS* AND RELATED ORGANISMS

It was reported by Van Heyningen[1] that growth of *Bacillus anthracis* was not inhibited on nutrient agar containing 50 μg of hematin per ml, although certain other spore-bearing aerobes were inhibited. Pearce and Powell[2] developed a selective medium for *B. anthracis* containing hematin and lysozyme. Preliminary work in this laboratory on the development of a selective medium for isolating *B. anthracis* indicated that quantitative recovery was dependent on the age of hematin solutions added to the medium. Freshly prepared alkaline hematin solutions were found to be inhibitory for Gram-positive organisms.

Hematin solutions were prepared by dissolving 400 μg of hemin (Eastman Kodak Company) per ml of 0.01 N NaOH and autoclaving for 30 minutes at 5 psi (hemin changes to hematin in the presence of alkali). A stock hematin solution was stored at 4°C and used periodically in test media to determine the minimum aging time required for full recovery of various test organisms. The test medium was prepared by adding either freshly prepared or aged hematin solutions to heart infusion agar (HIA-Difco) in a final concentration of 40 μg/ml (pH 7.4); the medium was used after an overnight preincubation period at 37°C. The effect of pH was evaluated by adjusting the media in a range of pH 6.6 to 8.2. The test inoculum was prepared by removing growth from a 24-hour HIA slant and suspending it in 0.06 M phosphate buffer. Dilutions of the suspension were adjusted to contain 10^3 organisms per ml and 0.1 ml was added to each plate (triplicate plates used and incubated at 37°C).

Recovery of Gram-positive organisms on the various media is shown in Table I. All of the *Bacillus* species tested, *Staphylococcus aureus*, and three *Streptococcus* strains were markedly inhibited in the presence of fresh hematin.

The inhibition of soil organisms was significantly increased, but *Escherichia coli*, *Aerobacter aerogenes*, *Pseudomonas aeruginosa* and four strains of *Pasteurella pestis* were not inhibited by freshly prepared hematin solutions. Further inhibition of Gram-positive organisms occurred when the concentration of fresh hematin was increased, but full recovery was obtained on media containing as much as 80 μg/ml of hematin aged for 2 months at 4°C. Recovery of some strains from media containing aged hematin was more than double the recovery from the HIA control. Fresh hematin solutions prepared with hematin from two different sources (Nutritional Biochemical Corp. and Eastman Kodak Co.) were equally inhibitory. A 14-day aging period of hematin was required for full recovery of *B. cereus* and *B. subtilis*; 3 to 4 weeks were required for *B. cereus* var. *mycoides*, *S. aureus*, and *B. anthracis*. Similar results were

obtained when nutrient agar was used in place of HIA. Changing the pH of the medium containing fresh hematin had no significant effect on recovery of test organisms. The exact composition of the alkaline hematin solution when freshly prepared and the changes that occurred during storage were not determined.

Kammerer[3] reported inhibition of many Gram-positive organisms (including B. anthracis) by mesohemin while Gram-negative organisms were generally not inhibited. He was also able to achieve complete inhibition of B. anthracis and B. megatherium with a 1:300 dilution (3333 μg/ml) of hematin (age not specified).

In summary, the results indicate that freshly prepared alkaline hematin solution is inhibitory for many Gram-positive organisms, including B. anthracis, and therefore its use in a selective medium for the isolation of B. anthracis is questionable. However, freshly prepared alkaline hematin has been found useful in a selective medium for Pasteurella pestis.[3] Further work on a selective medium for B. anthracis is in progress.

TABLE I. PER CENT RECOVERY OF VARIOUS GRAM-POSITIVE ORGANISMS ON MEDIA CONTAINING FRESH AND AGED HEMATIN[a]/

Test Organism[b]/	Number of Strains	Average Recovery as Per Cent of HIA Control[c]/	
		Aged Hematin[d]/	Fresh Hematin[e]/
Bacillus anthracis	10	95 to 163	0 to 20
Bacillus anthracis (spore susp)	1	90	0
Bacillus agri	1	107	2
Bacillus albolactus	1	139	23
Bacillus cereus	7	83 to 266	0 to 14
Bacillus cereus var. mycoides	4	78 to 240	0 to 4
Bacillus circulans	1	222	10
Bacillus graveolens	1	100	6
Bacillus lentimorbus	1	100	8
Bacillus megatherium	3	93 to 111	0 to 23
Bacillus polymyxa	1	238	1
Bacillus pumilus	1	100	0
Bacillus sphaericus	2	94 to 96	16 to 18
Bacillus sphaericus var. fusiformis	1	80	1
Bacillus subtilis	5	60 to 133	0 to 53
Bacillus thuringiensis	2	122 to 147	0 to 2
Staphylococcus aureus	1	111	3
Streptococcus faecalis	4	59 to 133	0 to 115

a. 40 μg/ml.
b. Grown for 24 hours at 37°C on HIA slant, except spore suspension which was stored in distilled water at 4°C.
c. HIA, heart infusion agar as 100% recovery control.
d. Alkaline hematin solution stored at 4°C for 2 months.
e. Medium containing fresh hematin was used within 24 hours after preparation.

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